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The Horace Brown Medal.

Forever in Focus: Researches in Malting and Brewing Sciences[+]

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+ Six presentations were made on the various facets of this work in 2019, in Manchester (March 19), Edinburgh (March 20), Dublin (March 21), Sutton Bonington (March 26), London (March 28) and Vienna (April 16).

Abstract

The paper reviews a career of more than forty years researching topics in malting and brewing science. Some themes attracted particularly close attention, namely the endosperm cell walls of barley, dimethyl sulphide, flavour stability, foam and the impact of beer on health. However the scope has been far broader than that. The underlying imperative was to pursue research that was close to application and always focussed on a specific need in the processes involved in the production of beer.

Key words: β-glucan, cell walls, dimethyl sulphide, flavour, foam, health, oxygen, stability,

Direction in Research

For the greater part of two centuries the study of beer has presented the opportunity for countless fundamental discoveries to have been made in science generally, findings of far more relevance than for their significance in beer alone (*1*). Indeed while some of the breakthroughs emerged from a fundamental process or quality need in the production of beer (e.g. the elucidation of pure yeast technology, *2*), in rather more instances the research involved was of a rather "purer" nature, by which I mean more a case of research for the sheer need to unravel the unknown as opposed to endeavours in pursuit of solving a problem. Examples of this would be the elucidation of the concept of pH (*3*).

At the time that I was directing the fundamental research program of BRF International in Nutfield, England, I wrote of the sequence of research, from pure (fundamental), through pre-competitive (strategic) to competitive (applied) (*4, 5*). For my part, irrespective of whether I was employed by a research station [Brewing Research (Foundation) (International; BR(F)(I)], a brewing company (Bass and latterly Sierra Nevada) or academia (the University of California, Davis), a fundamental question I have always asked prior to embarking on a research project (whether at the bench or more theoretical) was "might this having a meaningful application either directly or indirectly in malting or brewing?". If the answer was "no" then I would never pursue that project. I have spelled out the areas in which I believe that malting and brewing research should be focused (*4, 6,7, 8*) and I have highlighted one approach to project selection (*5*).

I have never felt there to be a shortage of intriguing topic areas that fall within these guidelines. An innate curiosity in diverse areas, then, has meant that I might fairly be accused of dipping into many topics without sufficiently dotting all the I's and crossing all the t's in any of them. The good news is that, hopefully, it has left plenty of scope for others (if they are so inclined) to fill in the gaps. I respectfully highlight some of the potential future research projects herein.

Having said that, there have been several themes that have garnered my attention more keenly than others and they will be readily identifiable as this discourse continues.

The cell walls of barley

The efficient degradation of the cell walls of the starchy endosperm of barley is critical if the brewer is not to encounter problems with reduced extract yields, solid-liquid separation challenges and increased risks from turbidity (*9, 10, 11*). However it can equally be highlighted that the soluble fibre derived from an incomplete digestion of the principle cell wall constituents of mixed-linkage β-glucan and (especially) arabinoxylan (pentosan) does lead to beer frequently being a good source of soluble fibre (*12, 13*), whilst the almost comprehensive digestion of β-glucan to oligosaccharides generates end products that we showed to be prebiotics (*14*).

My first foray into the subject of β-glucans was working alongside Hilary Martin in developing an enzyme-based method for quantifying the levels of the primary cell wall component in barley (*15, 16*).

At first the method was based on converting β-glucan to enzyme-assayable glucose using a cellulose preparation from *Trichoderma viride*. That a cellulose (endo- β-1,4-glucanase) is capable of completely converting mixed linkage β1,3; 1,4-glucan to glucose indicated that there must be β1-3 glucanase activity present and I purified and characterized an exo-acting enzyme from Trichoderma (*17*). We needed to eliminate amyloglucosidase, which would otherwise lead to erroneously high values as the contaminant would generate glucose from starch. This removal was effected using heat, however we found that some batches proved more intransigent to this procedure than did others, so we switched the enzyme source to *Penicillium funiculosum* which was much more reliable in this regard (*18*).

Reading around the topic of cell wall degradation at the time focused my attention on the work of Ian Preece in Edinburgh (*19*) and Bill Meredith in Winnipeg (*20*). Preece spoke of cytoclastic and cytolytic reactions, whilst Meredith intriguingly used the proteolytic enzyme papain to solubilize a proportion of gum. Of particular curiosity was a paper by Bendelow in 1973 (*21*), in which he wrote

We have evidence that the rate of release of beta-glucan from a protein-carbohydrate matrix in the cell wall is a limiting factor in modification and in the reduction of viscosity during mashing. And in this connection, the use of barley as an adjunct comes to mind. It appears that we must look for, and measure, some proteolytic enzyme that releases bound glucan for attack by the glucanases, and then incorporate high levels of this protease in new barley varieties - or modify the cell-wall structure.

I took in a short paper by Bob Scott (22) in which he discussed a heat-tolerant enzymic activity in malted barley that brought β-glucan into solution. With Ms Martin's meticulous technical support, I determined

to identify that enzyme (*15*). We confirmed that there is freely soluble β-glucan but demonstrated that there most definitely is an activity that enhances the solubilisation of glucan and that a proportion of this enzyme is already present in raw barley, thus indicating that it is not endo-β-glucanase (*15*). I gave it the name solubilase, which compatriots seemed to either love or hate. More solubilase is developed during germination (*23*).

We identified the enzyme from raw barley as being a carboxypeptidase acting as an esterase. Denise Baxter had previously tentatively suggested that carboxypeptidase might be involved in the degradation of the cell walls (*24*).

Joanne Moore pursued the solubilase story further and from her work we drew the conclusion that there are four different solubilase fractions in malt (*25*). One of these was confirmed as carboxypeptidase, with the others displaying activity as xylanase, ferulic acid esterase (*26*) and "general" esterase (not carboxypeptidase).

It was only later, in Davis, that my outstanding post-doctoral fellow Makoto Kanauchi and I were able to drill down on this story in depth. (Dr. Kanauchi and I have now collaborated for more than two decades.) Two main approaches were employed. The first was to grow *Trichoderma viride* on barley cell walls, the rationale being that the organism would synthesise the enzymes in the sequence that it needs them to attack the substrate (*27*). We found that there was an early release of xylanase, as well as of carboxypeptidase. Furthermore, it was shown that pentosan was released before β-glucan. The second approach was to treat isolated walls with a series of highly purified enzymes (kindly supplied to us by the

Novozymes research team in Davis) and to measure the extent to which β-glucan could be solubilized by them (*28*). Intriguingly it was shown that xylanase was the most powerful enzyme for solubilizing β-glucan, whereas endo-β-glucanase could not solubilize arabinoxylan. We also found that there was a small contribution from arabinofuranosidase, feruloyl esterase and acetoxylan esterase, but their effect could most clearly be seen using scanning electron microscopy, with a clear cleaning up of the surface of the walls, rather like the de-fuzzing of wool (*29*).

Based on these data, we proposed a model for the cell wall of barley (*30*), in which the pentosan is concentrated on the outside of the wall and the β-glucan on the inside. That glucanase could effect some release of glucan indicated that there was not a total shielding of the inner wall by the outer wall. The external presence of the arabinoxylan was consistent with the aforementioned cleaning up of the wall appearance by the esterases, it being known that ferulic acid and acetic acid are linked through ester bonds to the pentosan. Palmer had already suggested that pentosan was concentrated on the periphery of the wall (*31*).

Further relevant experimentation included that by Kuntz and I (*32*), showing that xylanase, arabinofuranosidase and carboxypeptidase develop before endo- β-glucanase in germinating barley. Furthermore, Scheffler and I showed, in mashing studies with high barley loadings and commercial enzymes, that xylanase worsened the viscosity situation at low doses, β-glucanase improved it, but that a mixture of xylanase and β-glucanase was the most effective way to deal with adjuncts as gauged by viscosity, wort separation rates and extract yield (*33*). This is readily rationalised by xylanase making the glucan more available for digestion.

To this day we have no notion of the bond(s) that are broken by carboxypeptidase in the solubilase action! Originally, we surmised that they were ester linkages between carboxyl groups in the protein that is known to be present in the wall and hydroxyl groups in the glucan. Tentatively we indicated that it may have something to do with the protein-rich middle lamella between adjacent cells. It would be good to find out.

I am also querying whether there needs to be a change to the model, following a poster presented at the European Brewery Convention Congress in Antwerp in 2019 (*34*). Langenaeken and colleagues from KU Leuven and Ghent used scanning electron microscopy to firmly place β-glucan on the outside of the wall, which is diametrically contrary to all the evidence that we employed to present our wall model. Perhaps what we are seeing here relates to the two populations of β-glucan to be found in the endosperm cell walls of barley, namely gum (water-soluble) and hemicellulose (not freely soluble) (*35, 36*). Possibly the minor component (the gum) is surface-located and readily released during the preparation of the wall fractions that we employed, thereby exposing the pentosan layer, which in turn envelops the hemicellulosic β-glucan component. It is also interesting that Langenaeken and colleagues firmly identify pectin as a minor component of the walls and this bears greater scrutiny in the context of the solubilase story.

Fig 1 presents the original model together with a variant that would account for the surface presence of *β*-glucan.

In addition to the detailed look at solubilase, we have also made some forays into the other enzymes that are responsible for breaking down cell walls. Using a radial diffusion assay for endo-β-glucanase (*37*), we investigated this enzyme and inter alia discovered that it might be partially stabilized and made more resistant to heat by reduced glutathione (38). That the end products of β-glucan degradation in malting and mashing are oligosaccharides rather than glucose was explained by the finding that the exo-glucanases that are present and capable of yielding glucose do not do so because of a poor affinity (high K_m) for the products of the endo-β-glucanase action and, furthermore, these enzymes develop relatively late during germination (*39*).

Despite the comprehensive digestion of β-glucan during malting and mashing, it is remarkable that the arabinoxylan is largely retained and not digested. There are certainly endo-xylanases in malt (*40*) as well as ferulic acid esterase (*41*) and acetoxylan esterases (*41, 42*). It is suggested that a primary reason for the inefficient breakdown of the pentosans is the presence of inhibitors in the grain (*40*).

Finally in this topic area, we proposed an enzyme-based method for measuring pentosan (*43*).

Dimethyl sulphide (DMS)

BRF had been the centre of excellence in the study of DMS, from the initial recognition of this molecule as a key contributor to the aromas of many lager beers (*44*) to the excellent investigations of Harry White, who got so close to understanding the nature of the malt-derived precursors of DMS (*45*). White

suggested that there were two precursors located in the embryo of germinated barley, the first of which could be broken down by heat (H) (particularly under alkaline conditions, A) to yield DMS and which he called HADMS, and the second of which was present in malts cured at temperatures in excels of 75°C and that was metabolizable by yeast into DMS, hence "active HADMS". He suggested that the former was S-methylmethionine (SMM) attached to additional peptide material but he could not identify the latter.

Dickenson (*46*) demonstrated that the heat-sensitive precursor was SMM per se and claimed that it did not yield any material that could be converted to DMS by yeast. However, at BRF we remained convinced that yeast is capable of producing DMS during fermentation. Brian Anness and I spent many months pondering this problem but repeatedly drew a blank when we fed yeast with a potential precursor. However, it was drawn to our attention that Zinder and Brock (*47*) had shown that a range of bacteria could reduce dimethyl sulfoxide (DMSO) to DMS. At the end of one of the tables of data in that paper they had also tested *Saccharomyces cerevisiae* and found some capability in this area. This led to our work demonstrating that yeast does indeed convert DMSO (which we showed to be present in malt, especially after curing) into DMS (*48*). I demonstrated that the enzyme involved was methionine sulfoxide reductase (*49*, see also *50*). This is an enzyme present in many eukaryotes which serves the function of recycling methionine sulfoxide to methionine and thereby restoring its functionality. The reducing power is supplied by the small redox protein thioredoxin, which cycles between an oxidized (dithiol) form and a reduced (sulphydryl) form. The electrons for the reduction of the dithiol form are supplied by NADPH in a reaction catalysed by thioredoxin reductase.

Thioredoxin serves as the electron donor for a range of cellular reactions. Amongst these is the reduction of ribonucleotides to deoxyribonucleotides, the building blocks of DNA. Accordingly, if a cell is actively growing and multiplying, there is likely an a priori drain on thioredoxin to feed DNA synthesis and less available to reduce sulfoxides (*51,52*). This fits with our observations that DMS production by yeast was less under warmer fermentation conditions and in nitrogen-replete worts (*53*). We also showed that there is an inhibitor of DMSO reduction in wort and identified it as methionine sulfoxide (*54*). Strictly speaking, I should not call it an inhibitor, but rather an "out-competing substrate". The K_m for methionine sulfoxide for methionine sulfoxide reductase is vastly lower than that for DMSO, so if methionine sulfoxide is present it will be preferentially handled by the enzyme. This is one of the reasons why there can be more DMSO reduction in a lager fermentation than an ale fermentation, because there is more methionine sulfoxide produced at higher kilning temperatures. The other reason for higher DMS production in lagers is the lower fermentation temperature (*55*).

There remained many sceptics who, observing that there is vastly more DMS in pitching wort than the finished beer, could not countenance that yeast was contributing any significant quantity of DMS. However, they were losing sight of the fact that there is enormous loss of DMS with the fermenter gases. Thus the final DMS level is a balance between that in wort which is not volatilised and that which is produced by yeast from DMSO. Dupire and colleagues used labelled DMSO to show that 80% of the DMS can originate in DMSO (*56*). We also did labelling studies to confirm that in fermentations where there is clearly less DMS at the end than at the start, there is nonetheless DMS in the finished beer that originated in DMSO (*57*).

What is also evident is that certain bacteria are mightily capable of reducing DMSO to DMS (*52*).

A final piece of the jigsaw was our demonstration that phenyl ethanol (PE) and phenyl ethyl acetate (PEA) mask the perception of DMS in beers and the detection of DMS character is through a balance of DMS, PE and PEA (*58*).

Gaps? I feel that a considerable amount of work could be detected to the DMSO reduction story. I think there remains more to discover about why the gene for the enzyme is expressed to a greater degree at lower fermentation temperatures, why more DMS is produced at higher pitching wort gravities and also at higher pH (*52*).

Flavour

In my laboratories in UC Davis we pursued several other studies on flavour issues, including the study of sulphur volatiles in addition to DMS (*59*). It seems to me that much more necessary information remains to be discovered about the control of some of these volatiles, notably the thiols and the mercaptans. However, there needs to be further delving into the production of methyl thioacetate, which Kanauchi and I showed to be produced by alcohol acetyl transferase, the same enzyme from yeast that yields the esters (*60*).

Donaldson et al (61) made some of the earliest inroads into establishing a meaningful lexicon to describe hop aroma. In relation to hop variety, there are some who suggest that one of the impacts on hop

aroma is the release of flavoursome substances (aglycones) from a binding to sugar. It has been suggested that this is effected by β-glucosidases from yeast, although Kanauchi and I found these to be intracellular (*62*)

Meanwhile, a degree of clarity was brought to the diversity of enzymes in ale and lager yeasts that are responsible for reducing diacetyl during fermentation and maturation, with a greater degree of complexity in lager yeast (*63*). Of course, for the longest time prolonged maturation of lager-based beers has been advocated, in part (but not entirely) to mop up the last traces of vicinal diketones and acetaldehyde. From the days of his employment within a brewing company that did not see the merits of prolonged aging of beer, the present author has been sceptical of its necessity. Laura Metrulas and colleagues used metabolomics to illustrate our inability to find significant changes in non-volatile molecules in beers during prolonged storage (*64*). [We had previously explored the use of this technique to address impacts of later and dry hopping (*65*).] I am extremely respectful of the fact that there are many who insist that prolonged lagering is beneficial, basing their conclusions on their own sensory perceptions. I am equally clear that we only examined a relatively narrow range of storage conditions. I insist that this territory represents a vast seam of research potential, insisting that it needs to be a combination of meticulous and detailed analytical chemistry with robust organoleptic approaches.

In the writing of this paper I was intrigued to find an account by Horace Brown of his findings on a tour to the United States (*66*). In discussing the production of lager beers, he referred to an accelerated process (vacuum-based) and made the following observation:

That a perfectly sound and saleable article can be produced by the vacuum system is an undeniable fact, but whether the longer storage and maturation of the beer brewed on the old system does not produce certain high class qualities which are lacking in the more quickly made and less matured vacuum beers can only be determined by a lengthy trial and by the competition of trade, which can alone decide which of the two is the fitter for survival. At present I hold my judgment on this question in suspense.

Flavour instability

Although we showed how brand identity can seriously confuse the perception of beer freshness (*67*), I remain convinced that the achievement of flavour-stable beer is the last major technical challenge facing brewers, important because staling for most beers seriously detracts from their drinkability.

Roy Parsons and I were the first to draw meaningful attention to a role for reactive oxygen species as a key underpinning cause of oxidative damage to wort and beer (*68*). In the pursuit of understanding how the development of such radicals could be prevented, we investigated a number of enzymes, notably superoxide dismutase (*69*, including soy beans as a source of a particularly heat-tolerant version of this enzyme*, 70*), catalase and peroxidases (*71, 72, 73, 74, 75*). Our work also highlighted to significance of minimizing the levels of certain metal ions in wort and beer, notably iron and copper (*76, 77, 78, 79, 80, 81*) and, as we later confirmed, manganese (*82*). In contrast to the focus of many, I have questioned the relative significance of lipoxygenase to flavour instability (*83, 84*).

Investigations over many years reinforced my opinion that flavour stability is a problem that should be addressed commercially "in reverse order", with a focus on beer in the trade first and then tracking back (*85, 86, 87*). Thus, I am at pains to emphasize absolutely that the two most important considerations should be the minimization of oxygen in the final package and the maintenance of beer at the lowest possible temperature (short of freezing) throughout storage and distribution. Only once this is assured is it worth paying attention to points upstream.

Mindful of the fact that many researchers are emphatic in their beliefs that there is much that can be done upstream to improve shelf life, I have felt it important to reemphasise the opinion first voiced by Meilgaard (*88*), that so much of the research on the sensory attributes of beer in relation to aging is substandard. To that end, I emphasize that the yardstick for flavour stability should be **time** to a detectable flavour change and not **intensity** of flavour change (*89, 90*). I am fully aware that I can be criticised for not having adhered to this in my earlier researches.

It is unquestionably the fact that oxygen is consumed upstream. Oxygen entering mashes causes an increase in colour, an attendant decrease in polyphenol levels, an increase in haze, reduced rates of wort separation and a decrease in the level of free thiols (*91*). In relation to this, our discovery of ascorbate peroxidase (*92*) and especially ascorbate oxidase (*93*) in malt has relevance. By the addition of ascorbate to mashes, the latter enzyme, which is remarkably heat resistant, can preferentially scavenge the oxygen and make for less thiol oxidation, less colour and higher polyphenol levels in the wort. For those convinced that brew house oxidation is important for flavour stability, herein lies one approach that is very much deserving of a lot more research. I also suggest that there is more to be done to build on the initial studies on other oxidases, notably oxalate oxidase (*94*) and thiol oxidase (*95*). In passing I

note that we suggested that a depletion of thiol oxidase during the storage of freshly kilned malt is a causative factor in the attendant improved brewhouse performance of that malt.

Foam

We can be confident that foam truly is a critical determinant of beer quality (*96, 97, 98*). We can be just as sure that problems with beer foam are far more likely to arise in the bar than in the brewery (*99*). Nonetheless it is critical that we have the fullest possible understanding of the chemistry and physics of beer in relation to foaming properties (*100, 101, 102, 103*).

Philip Slack and I used hydrophobic interaction chromatography to draw attention to hydrophobicity as a key feature of the polypeptides that afford the most stable foams (*104*). Subsequent studies showed that the proteins in the albumin fraction from the grain have more foam stabilizing capability than those that are derived from hordeins through partial hydrolysis (*105*), although the latter appear to have greater capability to enter into foam, thereby interfering with the ability of proteins such as lipid transfer protein and Protein Z to exert their stabilizing influence (*106*). This is a field of inquiry well worthy of further investigation, the logistical inference being that removal of hordein-derived fragments should lead to superior foaming performance in a beer. That would suggest that the enzyme prolyl endoproteinase, employed both as a haze-preventing agent and in the production of gluten-free beers, might actually be expected to **improve** foam stability. The observations made thus far in this area are contradictory (*103*).

Beer is, of course, a complex matrix and a plethora of substances can alternatively stabilize or inhibit foam (*107, 108*). One of the challenges is in relating studies made in model systems, using isolated or even non-native materials such as exogenous polypeptides, to what happens in beer poured into a glass. An example is pH, where a decrease in pH in the range 4.0 to 4.5 can variously lead to an increase or a decrease in perceived form performance (*108, 109*).

The reality is that foam is an extremely complex phenomenon, with the net foam performance attendant upon very many variables, both chemical and physical (*110*). Furthermore, the manifestation of foam is complex, with diverse aspects to it, including formation (nucleation or beading, which we modelled, *111*), retention (stability), texture and colour, and lacing (cling). Small wonder that a range of methods have been proposed for measuring foam, several which we have evaluated (*112, 113, 114*).

In terms of our own method development, Gordon Jackson and I long ago developed the lacing index procedure and applied it to assess some of the factors that influence the clingability of foam (*115, 116*).

I maintain, however, that the route to solving foam issues lies in the availability of robust methods to allow the investigator to determine whether a deficiency in perceived stability is due to a shortage of foam positives, presence of foam negatives or both. Chandley and I developed a test for assessing the level of foam positive polypeptides in beer (*117*). It was marketed commercially by BRFI and involves measuring protein in beer before and after removing the hydrophobic polypeptides on tiny columns of phenyl sepharose. The decrease in protein indicates the total amount of hydrophobic (foam-positive) polypeptide present. The problem is that there is a substantial amount of protein in beer and thus there

366 is a subtraction of two relatively large numbers to yield a rather smaller one, with the attendant
367 imprecision entailed. Nonetheless, others have used it to good effect. We did attempt to develop a
368 fluorescence-based approach to assessing hydrophobic polypeptides (*118*).

369

370 Rather more promising in my opinion are the approaches originally developed by Roy Cope and I (*119,*
371 *120*; see also *121*) and Kamini Dickie et al (*122*), both of which were explored in more detail by Goldberg
372 and Bamforth and myself (*123*). The first of these involves the addition of egg albumin to beer,
373 ultrafiltered beer and to a control alcohol solution to ascertain whether there is a shortage of
374 polypeptide or the presence of foam negative material. The second approach is the passage of beer
375 through a column containing lipid binding protein, the argument being that an increase in foam stability
376 attendant upon this treatment is indicative of there being foam inhibitors in the beer.

377

378 In our work we were able to demonstrate that many commercial beers contain substantial levels of
379 foam-negative materials. The likeliest source of these is the grist. We showed that there is foam
380 negative materials in all malts, and that the net contribution that these malts make to foam
381 performance is a balance between their level of foam-negative materials and foam-positive materials
382 (*124, 125*). Thus, we confirmed the long-standing view that wheat is a superior source of foam-positive
383 material than is barley (*124*). However, we were surprised to find that, contrary to established dogma
384 that crystal malts were foam-positive, they in fact can contain significant amounts of foam-negative
385 materials, at least some of which were oxidized fatty acids (*125*). The intriguing thing is that the crystal
386 malt possessed of the most foam-negative material gave beer with better foam stability than the crystal
387 malt that contained less foam-negative material (*124*). I suggest the following explanation, something
388 else worthy of further investigation: the foam-negative material from the crystal malt serves as an anti-

foam during fermentation. As such, it serves to preserve foam-positive material in the beer. Provided the foam-negative material is removed downstream (e.g. by filtration), the ensuing foam performance in the beer is superior. If, however the foam-negative material survives into the beer, it is damaging to foam performance in the glass.

Combe et al (*124*) confirmed that the best foam performance of all is from heavily roasted grain. Subsequently Emily Kultgen, Makoto Kanauchi and I demonstrated that the foam stabilising material from black malt is a very small molecule, featuring some peptide material and pyridyl entities produced during the roasting process (*126*). There is huge scope for further research here, as this material could represent a wonderful foam-promoting addition if it can be successfully separated from the coloured entities.

Beer, health and perceptions

One area that others in the brewing industry have been less than comfortable in me pursuing is that of beer (in moderation) as a component of a healthful lifestyle. The issue was not that they did not believe it to be true, but rather that the perception would be that a scientist working within the industry could not take a dispassionate approach to the matter. I have been assiduous in not using industry funds in any of my work and I have always been at pains to emphasize negatives as well as positives as I sought to (at the least) illustrate how beer is more than the equal of wine, for which beverage there seems not to have been the same reluctance to hold back (*127-137*). We have highlighted how wine is (incorrectly) perceived as a healthier option (*138, 139*). We have also highlighted the ignorance concerning matters

411 of beer and brewing that many people have (*140*). There should be no let-up in the education of the
412 drinking public.

413

414 In terms of specific research on health-related issues concerning beer, then we have included studies on
415 antioxidants (*141*), folate (*142*), minerals (*143*) especially silicate (*144*), as well as soluble fibre and
416 prebiotics as referred to earlier. Furthermore, we have explored the gliadin content of commercial beers
417 (*145*) and highlighted the merit of the enzyme prolylendoproteinase in producing gluten-free beer (*146*).
418 We employed an ELISA-based procedure to measure gliadin. There are those who insist that it is
419 insufficiently sensitive and are critical of its use in beer. This area needs extensive research, including
420 studies in conjunction with those in the medical profession.

421

422 We also did not fight shy of the matter of carbohydrates in beer in relation to health, discussing the
423 nonsense of the beer belly and the glycaemic index as it pertains to beer (*147*).

424

425 Downstream processing and haze

426

427 Bizarre as it may seem in the current climate of turbid beers, our researches have from time-to-time
428 dwelled on aspects to do with enhancing the colloidal stability of beer (*148, 149*). Indeed, one study
429 from 2007 demonstrated that there was a clear preference for bright beer over even the most marginal
430 of turbid brews (*150*). Perhaps such a study at the current time would yield a different outcome?

431

Of particular significance was the work of Michaela Miedl and I, in which we demonstrated that the key to precipitation of material in cold stabilization procedures is the lowness of the temperature rather than the time of storage (*151*). We also pursued model systems to extend the seminal work of Siebert on chill haze (*152*). Other relevant research concerned the efficiency of polyphenol adsorbents (*153, 154*), including the demonstration that PVPP has no impact on the flavour stability of beer (*155*). Finally, we identified unmodified barley endosperm as one of the causes of invisible hazes in beer – simultaneously failing in our efforts to get these problems referred to as "pseudo haze" (*156*).

Colour

My contributions to the matter of beer colour have included demonstration of the confusion that can arise in the non-trained taster's perceptions of flavour if the colour of a specific beer is modified (*157*), as well as confirming the weakness of colour methodology based on a single wavelength (*158*)

Ions and pH

Whilst it is customary to approach brewing and beer on a process or product basis, e.g. mashing, yeast, foam, stability etc, it can also be useful to address matters based on chemistry per se. For example, the approach taken in the review of matters of ionic equilibria and pH, influencing as they do diverse aspects of process and product performance (*159, 160*). There is much more scope for this approach.

Starch

Apart from failing to confirm the suggestion made elsewhere that limit dextrinase can be released in an active form by the thioredoxin system (*161*), my solitary contribution in the starch field has been a review (*162*). This certainly does not mean that I feel the area to be unfruitful. I believe, for example, that there is an urgent need to understand the interaction between barley variety, extent of modification and temperature in relation to starch gelatinization.

Genetic modification

While I was Director of Research at BRF International in the 1990's, John Hammond and his team made the pioneering studies into securing approval for the world's first genetically modified brewing yeast and we also explored opportunities for improving other brewing raw materials, notably barley (*163, 164*). I have long been convinced that sizeable take-up of genetically modified organisms in brewing would be dependent on there being a genuine and incontrovertible benefit for the customer and environment. More recently we were associated with our sister campus, UC Berkeley, in their development of yeasts that express genes that generate interesting flavours (*165*). There has already been commercial take up of some of these strains, one of the arguments in favour being that they may lower the environmental footprint of certain crops.

Microbiology

Regarding matters microbiological, then Nick Bokulich made an extensive contribution in his time in my laboratory (*166, 167, 168*). Of note was the extensive examination of the evolution of the diverse microflora in the production of "wild" beers (*169*). In relation to the production of sour beers, one interesting approach would be the use of a single organism capable of producing both alcohol and souring quantities of acid, such as Lachancea (*170*).

Enzymology

As someone who emerged from his graduate studies as an enzymologist, it is perhaps no surprise that much of the research referred to above is at the enzyme level. In terms of generalities of enzymes in brewing, then go to references *171-178*.

Business of beer

I started this discourse by emphasizing matters of project selection and the imperative of ensuring relevance in one's research focus. It is essential for anyone intent on pursuing brewing research to understand the business and its trajectories. I have been fortunate to be in collaboration with Ignazio Cabras on such matters (*179, 180*).

When I first joined the brewing industry in 1978 there are many things I would not have predicted: the growth of the (so-called) craft sector; the love of immensely turbid beers; beers possessed of bizarre ingredients; the degree of in-line sophistication; and more. However, the fundamental brewing paradigm of barley-malt-milled malt-mash-lauter-boil-clarify-cool-ferment-mature-stabilize-package has remained unaltered. From time to time I have suggested alternatives (*6, 181, 182, 183, 184, 185*). I find it very hard to ever countenance beer being produced from a bland alcoholic base (*186*), despite its potential environmental advantage (*187*). However in an era when there is such interest in hard seltzers…quo vadis?

References

1. Anderson, R.G. (1992) The pattern of brewing research: a personal view of the history of brewing in the British Isles. *J. Inst. Brew*. *98*, 85-109. https://doi.org/10.1002/j.2050-0416.1992.tb01095.x
2. Claussen, N.H. (1904) On a Method for the Application of Hansen's Pure Yeast System in the Manufacturing of Well-Conditioned English Stock Beers. *J. Inst. Brew*. *10*, 308-331. https://doi.org/10.1002/j.2050-0416.1904.tb04656.x
3. Sörensen, S. P. L. (1909). Enzymstudien. II. Mitteilung. Über die Messung und die Bedeutung der Wasserstoffionenkoncentration bei enzymatischen Prozessen. *Biochem. Zeit*. *21*, 131–304.
4. Bamforth, C.W. (1993) The worldwide role of BRF International. *Ferment*, *6*, 333- 338
5. Bamforth, C.W. (1995) Economic assessment of research and development. *Tech. Quart. Mast. Brew. Assoc. Amer*. *32*, 132-137

6. Bamforth, C.W. (2000) Brewing and brewing research: past, present and future. *J. Sci. Food Ag. 80*, 1371-1378. doi:10.1002/1097-0010(200007)80:9<1371::AID-JSFA654>3.0.CO;2-K
7. Bamforth, C.W. (2001) The need for research to satisfy future brewing needs. *Pauls Brewing Room Book*
8. Bamforth, C., ed (2006) *Brewing: New Technologies*. Woodhead Publishing
9. Bamforth, C.W. (1982) Barley β-glucans: their role in malting and brewing. *Brew. Dig. 57 (6)*, 22-27, 35.
10. Bamforth, C.W. (1994) β-Glucan and β-glucanases in malting and brewing: practical aspects. *Brew. Dig. 69 (5),* 12-16, 21
11. Bamforth, C.W. (2010) The enzymology of cell wall breakdown during malting and mashing: an overview. *Tech. Quart. Mast. Brew. Assoc. Amer*, doi:10.1094/TQ-47-1-0309-01
12. Bamforth, C.W. and Gambill, S.C. (2007) Fiber and putative prebiotics in beer. *J. Am. Soc. Brew. Chem*. 65, 67-69. doi:10.1094/ASBCJ-2007-0306-01
13. Kanauchi, M., Ishikura, W. and Bamforth, C.W. (2011) β-Glucans and pentosans and their degradation products in commercial beers. J Inst Brew, 117, 120-124
14. Kanyer, A.J., Bornhorst, G.M., Marco, M.L. and Bamforth, C.W. (2017). Is beer a source of prebiotics? *J. Inst. Brew., 123*, 361-365. https://doi.org/10.1002/jib.439
15. Bamforth, C.W., Martin, H.L. and Wainwright, T. (1979) A role for carboxypeptidase in the solubilization of barley β-glucan. *J. Inst. Brew., 85*, 334-338. https://doi.org/10.1002/j.2050-0416.1979.tb03937.x
16. Martin, H.L. and Bamforth, C.W. (1981) An enzymic method for the measurement of total and water-soluble β-glucan in barley. *J. Inst. Brew., 87*, 88-91. https://doi.org/10.1002/j.2050-0416.1981.tb03994.x

17. Bamforth, C.W. (1980) The adaptability, purification and properties of exo-1,3-β-glucanase from the fungus Trichoderma reesei. *Biochem. J., 191*, 863-866. DOI: https://doi.org/10.1042/bj1910863
18. Bamforth, C.W. (1983) Penicillium funiculosum as a source of β-glucanase for the estimation of barley β-glucan. *J. Inst. Brew., 89*, 391-392. https://doi.org/10.1002/j.2050-0416.1983.tb04212.x
19. Preece, I.A., Ashworth, A.S. and Hunter, A.D. (1950) Cytolysis in germinating barley: 1. Some barley and malt polysaccharides. *J. Inst. Brew., 56*, 33-40. https://doi.org/10.1002/j.2050-0416.1950.tb01518.x
20. Bass, E.J. and Meredith, W.O.S. (1955) Cytolytic Enzymes in Germinating Barley A Review of Current Research. *Proc. Am. Soc. Brew. Chem. 13*, 11-17. https://doi.org/10.1080/00960845.1955.12006443
21. Bendelow, V.M. (1973). Current research on quality improvement of Canadian malting barley. Tech. Quart. Mast. Brew. Assoc. Amer. 10, 53-55
22. Scott, R.W. (1972) Solubilization of β-glucan during mashing. *J. Inst. Brew. 78*, 411-412. https://doi.org/10.1002/j.2050-0416.1972.tb03472.x
23. Bamforth, C.W. and Martin, H.L. (1981) The development of β-glucan solubilase during barley germination. *J. Inst. Brew*., 87, 81-84. https://doi.org/10.1002/j.2050-0416.1981.tb03991.x
24. Baxter, E.D. (1978) Purification and properties of malt carboxypeptidases attacking hordein. *J. Inst. Brew*., 84, 271-275. https://doi.org/10.1002/j.2050-0416.1978.tb03886.x
25. Bamforth, C.W., Moore, J., McKillop, D., Williamson, G. and Kroon, P.A. (1997) Enzymes from barley which solubilize β-glucan. *Proc. Eur. Brew. Conv. Cong., Maastricht*, 75-82
26. Moore, J., Bamforth, C.W., Kroon, P.A., Bartolome, B. and Williamson, G. (1996) Ferulic acid esterase catalyses the solubilization of β-glucans and pentosans from the starchy endosperm cell walls of barley. *Biotech. Lett. 18*, 1423-1426. https://doi.org/10.1007/BF00129347

27. Kanauchi, M. and Bamforth, C.W. (2001) Growth of *Trichoderma viride* on crude cell wall preparations from barley. *J. Ag. Food Chem., 49*, 883-887. https://doi.org/10.1021/jf001001d
28. Kanauchi, M. and Bamforth, C.W. (2001) The release of β-glucan from cell walls of the starchy endosperm of barley. *Cereal Chem., 78*, 121-124. http://dx.doi.org/10.1094/CCHEM.2001.78.2.121
29. Kanauchi, M. and Bamforth, C.W. (2002) Enzymic digestion of walls purified from the starchy endosperm of barley, *J. Inst. Brew. 108*, 73-77. https://doi.org/10.1002/j.2050-0416.2002.tb00127.x
30. Bamforth, C.W. and Kanauchi, M. (2001) A simple model for the cell wall of the starchy endosperm in barley, *J. Inst. Brew., 107*, 235-240. https://doi.org/10.1002/j.2050-0416.2001.tb00095.x
31. Palmer, G. H. (1975) Influence of endosperm structure on extract development. *Proc. Am. Soc. Brew. Chem. 33*, 174–180.
32. Kuntz, R.J. and Bamforth, C.W. (2007) Time Course for the development of Enzymes in Barley. *J. Inst. Brew. 113*, 196-205. https://doi.org/10.1002/j.2050-0416.2007.tb00276.x
33. Scheffler, A. and Bamforth, C.W. (2005) Exogenous β-Glucanases and Pentosanases and their impact on mashing. *Enz. Microb. Tech*. *36*, 813-817. https://doi.org/10.1016/j.enzmictec.2005.01.009
34. Langenaeken, N.A. (2019) KU Leuven, personal communication.
35. Martin, H.L. and Bamforth, C.W. (1980) The relationship between β-glucan solubilase, barley autolysis and malting potential. *J. Inst. Brew. 86*, 216-221. https://doi.org/10.1002/j.2050-0416.1980.tb06869.x

585 36. Lee, Y.T. and Bamforth, C.W. (2009) Variations in Solubility of Barley β-Glucan during Malting
586 and Impact on levels of β-Glucan in Wort and Beer. *J. Am. Soc. Brew. Chem. 67*, 67-71.
587 https://doi.org/10.1094/ASBCJ-2009-0226-01
588 37. Martin, H.L. and Bamforth, C.W. (1983) Application of a radial diffusion assay for the
589 measurement of β-glucanase in malt. *J. Inst. Brew., 89*, 34-37. https://doi.org/10.1002/j.2050-
590 0416.1983.tb04140.x
591 38. Bamforth, C.W. and Martin, H.L. (1983) The degradation of β-glucan during malting and
592 mashing: the role of β-glucanase. *J. Inst. Brew., 89,* 303-307. https://doi.org/10.1002/j.2050-
593 0416.1983.tb04190.x
594 39. Kanauchi, M. and Bamforth, C.W. (2008) The relevance of different enzymes for the hydrolysis
595 of β-glucans in malting and mashing. *J. Inst. Brew, 114*, 224–229.
596 https://doi.org/10.1002/j.2050-0416.2008.tb00332.x
597 40. Kanauchi, M., Chijimi, A., Ohnishi-Kameyama, M. and Bamforth, C.W. (2013) An investigation of
598 two xylan-degrading enzymes and a novel xylanase inhibitor in malted barley. *J. Inst. Brew., 119*,
599 32-40. https://doi.org/10.1002/jib.64
600 41. Sun, A., Faulds, C.B. and Bamforth C.W. (2005). Barley contains two cationic acetylxylan
601 esterases and one anionic feruloyl esterase. *Cereal Chem. 82*, 621-625.
602 http://dx.doi.org/10.1094/CC-82-0621
603 42. Ward, R.E. and Bamforth, C.W. (2002) Esterases in barley and malt. *Cereal Chem. 79*, 681-686.
604 http://dx.doi.org/10.1094/CCHEM.2002.79.5.681
605 43. Kanauchi, M. and Bamforth, C.W. (2003) Use of Xylose dehydrogenase from Trichoderma viride
606 in an enzymic method for the measurement of pentosan in barley. *J. Inst. Brew. 109*, 203- 207.
607 https://doi.org/10.1002/j.2050-0416.2003.tb00160.x

44. Anderson, R.J., Clapperton, J.F., Crabb, D. and Hudson, J.R. (1975) Dimethyl sulphide as a feature of lager flavour. *J. Inst. Brew. 81*, 208-213. https://doi.org/10.1002/j.2050-0416.1975.tb03679.x
45. White, F.H. and Wainwright, T. (1976) Isolation and partial characterization of the dimethyl sulphide precursor in green malt and its effect on beer dimethyl sulphide levels. *J. Inst. Brew. 82*, 292-296. https://doi.org/10.1002/j.2050-0416.1976.tb03777.x
46. Dickenson, C.J. (1979) Identification of the dimethyl sulphide precursor in malt. *J. Inst. Brew. 85*, 329-333. https://doi.org/10.1002/j.2050-0416.1979.tb03936.x
47. Zinder, S.H. and Brock, T.D. (1978) Dimethyl sulphoxide reduction by micro-organisms. *J. Gen. Microbiol. 105*, 335-42. DOI: 10.1099/00221287-105-2-335
48. Anness, B.J., Bamforth, C.W. and Wainwright, T (1979) The measurement of dimethyl sulfoxide in barley and malt and its reduction to dimethyl sulfide by yeast. *J. Inst. Brew., 85*, 346-349. https://doi.org/10.1002/j.2050-0416.1979.tb03940.x
49. Bamforth, C.W. (1980) Dimethyl sulfoxide reductase of Saccharomyces spp. *FEMS Micro. Lett. 7*, 55-59. DOI: 10.1111/j.1574-6941.1980.tb01576.x
50. Sugai, T., Kanauchi, M. and Bamforth, C.W. (2017) Characterization of Dimethyl Sulfoxide Reductase from Brewing Yeast. *J. Inst. Brew. 123*, 337-346. https://doi.org/10.1002/jib.435
51. Bamforth, C.W. and Anness, B.J. (1981) The role of dimethyl sulfoxide reductase in the formation of dimethyl sulfide during fermentations. *J. Inst. Brew. 87*, 30-34. https://doi.org/10.1002/j.2050-0416.1981.tb03981.x
52. Anness, B.J. and Bamforth, C.W. (1982) Dimethyl sulfide - a review. *J. Inst. Brew. 88*, 244-252. https://doi.org/10.1002/j.2050-0416.1982.tb04101.x
53. Gibson, R.M., Large, P.J. and Bamforth, C.W. (1985) The influence of assimilable nitrogen compounds in wort on the ability of yeast to reduce dimethyl sulfoxide. *J. Inst. Brew., 91*, 401-405. https://doi.org/10.1002/j.2050-0416.1985.tb04364.x

54. Gibson, R.M., Large, P.J., Anness, B.J. and Bamforth, C.W. (1983) The identity of an inhibitor in wort of dimethyl sulfoxide reductase from yeast. *J. Inst. Brew., 89*, 215-218. https://doi.org/10.1002/j.2050-0416.1983.tb04170.x
55. Bamforth, C.W. (2014) Dimethyl sulfide – Significance, Origins and Control. *J. Am. Soc. Brew. Chem. 72*, 165-168. DOI: 10.1094/ASBCJ-2014-0610-01
56. Leemans, C., Dupire, S., and Macron, J.-Y. (1993) Relation between wort DMSO and DMS concentration in beer. *Proc. Eur. Brew. Conv. Cong., Oslo*, 709-716
57. Gibson, R.M., Large, P.J. and Bamforth, C.W. (1985) The use of radioactive labeling to demonstrate the production of dimethyl sulfide from dimethyl sulfoxide during fermentation of wort. *J. Inst. Brew.* 91, 397-400. https://doi.org/10.1002/j.2050-0416.1985.tb04363.x
58. Hegarty, P.K., Parsons, R., Bamforth, C.W. and Molzahn, S.W. (1995) Phenyl ethanol - a factor determining lager character. *Proc. Eur. Brew. Conv. Cong., Brussels*, 515-522
59. Miracle, R.E. Ebeler, S.E. and Bamforth, C.W. (2005). The measurement of sulfur-containing aroma compounds in samples from production-scale brewery operations. *J. Am. Soc. Brew. Chem. 63*, 129-134. https://doi.org/10.1094/ASBCJ-63-0129
60. Bamforth, C.W. and Kanauchi, M. (2003) Use of novel assays to indicate that O-esters and S-esters are produced by the same enzyme in brewing yeast. *FEMS Micro. Lett. 228*, 111-113. https://doi.org/10.1016/S0378-1097(03)00742-0
61. Donaldson, B.A., Bamforth, C.W. and Heymann, H. (2012) Sensory Descriptive Analysis and Free-Choice Profiling of Thirteen Hop Varieties as Whole Cones and After Dry Hopping of Beer. J. *Am. Soc. Brew. Chem. 70*, 176-181. https://doi.org/10.1094/ASBCJ-2012-0710-01
62. Kanauchi, M. and Bamforth, C.W. (2012) β-Glucoside hydrolyzing enzymes from ale and lager strains of brewing yeast. *J. Am. Soc. Brew. Chem. 70*, 303-307. https://doi.org/10.1094/ASBCJ-2012-1012-02

63. Bamforth, C.W. and Kanauchi, M. (2004) Enzymology of Vicinal Diketone Reduction in Brewer's Yeast. *J Inst Brew, 110*, 83-93. https://doi.org/10.1002/j.2050-0416.2004.tb00187.x

64. Metrulas, L.K., McNeil, C., Slupsky, C.M. and Bamforth, C.W. (2019). The application of metabolomics to ascertain the significance of prolonged maturation in the production of lager-style beers. *J. Inst. Brew, 125*, 242-249. https://doi.org/10.1002/jib.557

65. Spevacek, A.R., Benson, K.H., Bamforth, C.W. and Slupsky, C.M. (2016) Beer Metabolomics: Molecular Details of the Brewing Process and the Differential Effects of Late and Dry Hopping on Yeast Purine Metabolism. *J. Inst. Brew. 122*, 21-28. https://doi.org/10.1002/jib.291

66. Brown, H.T. (1897) On some Recent Advances in Brewing in the United States. *J. Inst. Brew. 3,* 467-480. https://doi.org/10.1002/j.2050-0416.1897.tb00325.x

67. Stephenson, W.H. and Bamforth, C.W. (2002) The impact of lightstruck and stale character in Beers on their perceived Quality: A Consumer Study. *J. Inst. Brew. 108*. 406-409. https://doi.org/10.1002/j.2050-0416.2002.tb00568.x

68. Bamforth, C.W. and Parsons, R. (1985) New procedures to improve the flavor stability of beer. *J. Am. Soc. Brew. Chem. 43*, 197-202. https://doi.org/10.1094/ASBCJ-43-0197

69. Bamforth, C.W. (1983) Superoxide dismutase in barley. *J. Inst. Brew. 89*, 420-423. https://doi.org/10.1002/j.2050-0416.1983.tb04218.x

70. Clarkson, S.P., Large, P.J. and Bamforth, C.W. (1989) Purification of a cyanide-sensitive superoxide dismutase from soybeans: a food-compatible enzyme preparation. *J. Sci. Food. Agric. 48*, 87-97. https://doi.org/10.1002/jsfa.2740480110

71. Bamforth, C.W., Clarkson, S.P. and Large, P.J. (1991) The relative importance of polyphenol oxidase, lipoxygenase and peroxidases during wort oxidation. *Proc. Eur. Brew. Conv. Cong., Lisbon,* 617-624

72. Clarkson, S.P., Large, P.J. and Bamforth, C.W. (1992) Oxygen-scavenging enzymes in barley and malt and their effects during mashing. *J. Inst. Brew. 98*, 111-115. https://doi.org/10.1002/j.2050-0416.1992.tb01096.x
73. Clarkson, S.P., Large, P.J. and Bamforth, C.W. (1992) A two-substrate kinetic study of peroxidase cationic isoenzymes in barley malt. *Phytochem*. *31*, 743-749. https://doi.org/10.1016/0031-9422(92)80005-Y
74. Antrobus, C.J., Large, P.J. and Bamforth, C.W. (1997) Changes in the cationic isoenzymes of peroxidase during the malting of barley. I Tissue location studies. *J. Inst. Brew. 103*, 227-231. https://doi.org/10.1002/j.2050-0416.1997.tb00949.x
75. Antrobus, C.J., Large, P.J. and Bamforth, C.W. (1997) Changes in the cationic isoenzymes of peroxidase during the malting of barley. II The effect of gibberellic and abscisic acids. *J. Inst. Brew. 103*, 233-237. https://doi.org/10.1002/j.2050-0416.1997.tb00950.x
76. Bamforth, C.W. (1986) Beer flavor stability. *Brewer, 72*, 48-51
77. Bamforth, C.W., Boulton, C.A., Clarkson, S.P. and Large, P.J. (1988) The effects of oxygen on brewery process performance. *Proc. Inst. Brew. Conf., Aust. NZ Section*, 211-219
78. Clarkson, S.P., Large, P.J., Hegarty, P.K. and Bamforth, C.W. (1989) Oxygen radicals - their influence on process performance and product quality. *Proc. Eur. Brew. Conv. Cong., Zurich*, 267-274
79. Bamforth, C.W., Muller, R.E. and Walker, M.D. (1993) Oxygen and oxygen radicals in malting and brewing: a review. *J. Am. Soc. Brew. Chem. 51*, 79-88. https://doi.org/10.1094/ASBCJ-51-0079
80. Bamforth, C.W., Hughes, P.S., Muller, R.E., Walters, M.T., Antrobus, C.J. and Large, P.J. (1996) Oxidation in malting and brewing: assessment, implication and elimination. *Proc. Int. Brew. Tech. Conf. Harrogate*, 267-276

81. Bamforth, C.W. (2001) Oxido-reduction processes and active forms of oxygen in aqueous systems. *Cere. Biotech., 26*, 149-154
82. Porter, J.R. and Bamforth, C.W. (2016) Manganese in brewing raw materials, disposition during the brewing process and impact on the flavor instability of beer. *J. Am. Soc. Brew. Chem. 74*, 87-90. https://doi.org/10.1094/ASBCJ-2016-2638-01
83. Bamforth, C.W. (1999) Enzymic and non-enzymic oxidation in the brewhouse; a theoretical consideration. *J. Inst. Brew. 105*, 237-242. https://doi.org/10.1002/j.2050-0416.1999.tb00025.x
84. Biawa, J.-P. and Bamforth, C.W. (2002) A two-substrate kinetic analysis of lipoxygenase in malt. *J. Cer. Sci. 35*, 95-98. https://doi.org/10.1006/jcrs.2001.0403
85. Bamforth, C.W. (1999) The science and understanding of the flavour stability of beer: a critical assessment. *Brau. Int. 17*, 98-110
86. Bamforth, C.W. (2004) A critical control point analysis for flavor stability of beer. *Tech. Quart. Mast. Brew. Assoc. Amer. 41*, 97-103.
87. Bamforth, C.W. (2004) Fresh Controversy: Conflicting Opinions on Beer Staling. *Proc. Conv. IGB Asia-Pacific*, 63-73
88. Meilgaard, M. (2001) Effects on Flavour of Innovations in Brewery Equipment and Processing: A Review. *J. Inst. Brew. 107*, 271-286. https://doi.org/10.1002/j.2050-0416.2001.tb00098.x
89. Kanauchi, M. and Bamforth, C.W. (2018) A Challenge in the study of flavour instability. *Brew. Sci. 71*, 74-76.
90. Bamforth, C.W. (2017) Practical Guides for Beer Quality: Freshness. ASBC Handbook Series. ASBC, St Paul MN
91. Stephenson, W.H., Biawa, J.P., Miracle, R.E. and Bamforth, C.W. (2003) Laboratory-scale studies of the impact of oxygen on mashing. *J. Inst. Brew., 109*, 273- 283. https://doi.org/10.1002/j.2050-0416.2003.tb00168.x

92. Kanauchi, M. and Bamforth, C.W. (2013) Ascorbate peroxidase in malted barley. *J. Am. Soc. Brew. Chem. 71*, 97-102. https://doi.org/10.1094/ASBCJ-2013-0103-01
93. Kanauchi, M., Simon, K.J. and Bamforth, C.W. (2014) Ascorbic acid oxidase in barley and malt and its possible role during mashing. *J. Am. Soc. Brew. Chem. 72*, 30-35. DOI: 10.1094/ASBCJ-2014-0120-01
94. Kanauchi, M., Milet, J. and Bamforth, C.W. (2009) Oxalate and Oxalate Oxidase in Malt. *J. Inst. Brew. 115,* 232-237. https://doi.org/10.1002/j.2050-0416.2009.tb00374.x
95. Bamforth, C.W., Roza, J.R and Kanauchi, M. (2009) Storage of malt, thiol oxidase and brewhouse performance. *J. Am. Soc. Brew. Chem. 67*, 89-94. https://doi.org/10.1094/ASBCJ-2009-0219-01
96. Bamforth, C.W. (2000) Perceptions of beer foam. *J. Inst. Brew. 106*, 229-238. https://doi.org/10.1002/j.2050-0416.2000.tb00062.x
97. Smythe, J.E., O'Mahony, M. and Bamforth, C.W. (2002) The Impact of the Appearance of Beer on its Perception. *J. Inst. Brew.* 108, 37-42. https://doi.org/10.1002/j.2050-0416.2002.tb00120.x
98. Smythe, J.E and Bamforth, C.W. (2003) The path analysis method of eliminating preferred stimuli (PAMEPS) as a means to determine foam preferences for lagers in European judges based upon image assessment. *Food Qual. Pref. 14*, 567- 572. https://doi.org/10.1016/S0950-3293(02)00133-7
99. Bamforth, C.W. (2012) Practical Guides for Beer Quality: Foam. ASBC Handbook Series. ASBC, St Paul MN
100. Bamforth, C.W. (1985) The foaming properties of beer. *J. Inst. Brew. 91*, 370-383. https://doi.org/10.1002/j.2050-0416.1985.tb04359.x
101. Bamforth, C.W. (1993) Recent progress in our understanding of beer foam. *Cerev. Biotech. 18*, 49-53

102. Bamforth, C.W. (1999) Bringing matters to a head: the status of research on beer foam. *Proc. Eur. Brew. Conv. Foam Symp., Amsterdam*, 10-23

103. Evans, D. E. and Bamforth, C. W. (2009) Beer foam: achieving a suitable head. In: *Beer: A Quality Perspective*, C. W. Bamforth, Ed., Academic Press: Burlington MA, 2009, pp. 1-60

104. Slack, P.T. and Bamforth, C.W. (1983) The fractionation of polypeptides from barley and beer by hydrophobic interaction chromatography: the influence of their hydrophobicity on foam stability. *J. Inst. Brew. 89*, 397-401. https://doi.org/10.1002/j.2050-0416.1983.tb04214.x

105. Kapp, G.R. and Bamforth, C.W. (2002) The foaming properties of proteins isolated from barley. *J. Sci. Food Ag. 82*, 1276-1281. https://doi.org/10.1002/jsfa.1177

106. Bamforth, C.W. and Milani, C. (2004). The foaming of mixtures of albumin and hordein protein hydrolysates in model systems. *J. Sci. Food Ag. 84*, 1001-1004. https://doi.org/10.1002/jsfa.1749

107. Bamforth, C.W., Canterranne, E., Chandley, P. and Onishi, A. (1993) The molecular interactions of beer foam. *Proc. Eur. Brew. Conv. Cong., Oslo*, 331-340

108. Bamforth, C.W. and Kanauchi, M (2003) Interactions between polypeptides derived from barley and other beer components in model foam systems. *J. Sci. Food Ag. 83*, 1045- 1050. https://doi.org/10.1002/jsfa.1503

109. Bamforth, C., Kalathas, A., Maurin, Y. and Wallin, C. (2008) Some factors impacting beer foam. *Tech. Quart. Mast. Brew. Assoc. Amer. 45*, 332-336

110. Bamforth, C.W. (2004) The relative significance of physics and chemistry for beer foam excellence: theory and practice. *J. Inst. Brew. 110*, 259-266. https://doi.org/10.1002/j.2050-0416.2004.tb00620.x

111. Lynch, D.M and Bamforth, C.W. (2002) Measurement and Characterization of Bubble Nucleation in Beer. *J. Food Sci. 67*, 2696-2701. https://doi.org/10.1111/j.1365-2621.2002.tb08801.x

112. Hung, J.K.S., Wallin, C.E. and Bamforth, C.W. (2005) An evaluation of an automated procedure for measuring beer foam stability. *Tech. Quart. Mast. Brew. Assoc. Amer. 42*, 178-183

113. Roza, J.R., Wallin, C.E. and Bamforth, C.W. (2006) A comparison between the instrumental measurement of head retention/lacing and perceived foam quality. *Tech. Quart. Mast. Brew. Assoc. Amer. 43,* 173-176

114. Wallin, C.E., DiPietro, M.B., Schwarz, R.W. and Bamforth C.W. (2010). A comparison of three methods for the assessment of foam stability of beer. *J. Inst. Brew. 116*, 78-80. https://doi.org/10.1002/j.2050-0416.2010.tb00401.x

115. Jackson, G. and Bamforth, C.W. (1982) The measurement of foam lacing. *J. Inst. Brew. 88*, 378-381. https://doi.org/10.1002/j.2050-0416.1982.tb04126.x

116. Bamforth, C.W. and Jackson, G. (1983) Aspects of foam lacing. *Proc. Eur. Brew. Conv. Cong., London*, 331-338

117. Bamforth, C.W. (1995) Foam: method, myth or magic? *Brewer*, 91, 396-399

118. Bamforth, C.W., Kapp, G.R. and Smythe, J.E. (2001) The measurement of hydrophobic polypeptides in beer using the fluorochrome 1-anilino-8-naphthalenesulfonate. *Food Chem. 75*, 377-383. https://doi.org/10.1016/S0308-8146(01)00222-9

119. Bamforth, C.W. and Cope, R. (1985) Original approaches to improving the foam of beer. *Proc. Eur. Brew. Conv. Cong., Helsinki*, 515-522

120. Bamforth, C.W. and Cope, R. (1987) Egg albumen as a source of foam polypeptide in beer. *J. Am. Soc. Brew. Chem. 45*, 27-32. https://doi.org/10.1094/ASBCJ-45-0027

121. St John Coghlan, D., Woodrow, J., Bamforth, C.W. and Hinchliffe, E. (1992) Polypeptides with enhanced foam potential. *J. Inst. Brew. 98*, 207-213. https://doi.org/10.1002/j.2050-0416.1992.tb01106.x

122. Dickie, K.H., Cann, C., Norman, E.C., Bamforth, C.W. and Muller, R.E. (2001) Foam-negative materials*. J. Am. Soc. Brew. Chem. 59*, 17-23. https://doi.org/10.1094/ASBCJ-59-0017

123. Goldberg, J.R. and Bamforth, C.W. (2010) Analyzing foam instability in commercial beers*. J. Am. Soc. Brew. Chem. 68*, 57-62. https://doi.org/10.1094/ASBCJ-2010-0121-01

124. Combe, A.L., Ang, J.K. and Bamforth, C.W. (2013) Positive and negative impacts of specialty malts on beer foam: A comparison of various cereal products for their foaming properties. *J. Sci. Food Ag. 93*, 2094-2101. https://doi.org/10.1002/jsfa.6117

125. Ang, J.K. and Bamforth, C.W. (2014) Foam inhibitors from specialty malts. *J. Inst. Brew. 120*, 193-200. https://doi.org/10.1002/jib.141

126. Kanauchi, M., Kultgen, E. and Bamforth, C. (2019) Low molecular weight materials from heavily roasted barley and malt with strong foam-stabilising potential*. J. Inst. Brew. 125*, 39-46. https://doi.org/10.1002/jib.538

127. Bamforth, C.W. (2002) Nutritional Aspects of Beer – A Review. *Nut. Res. 22*, 227-237. https://doi.org/10.1016/S0271-5317(01)00360-8

128. Bamforth, C.W. (2004) Beer: Health and Nutrition, Blackwell, Oxford

129. Bamforth, C.W (2004) An ungrateful subject? Differences of opinion on beer. *Brew. Guard. 133 (1),* 20-23

130. Bamforth, C.W. (2004) Teaching Brewing in a Passionate Society. *Proc Conv. IGB Asia-Pacific*, 50-53

131. Bamforth, C.W. (2007) Beer as liquid bread. *Tech. Quart. Mast. Brew. Assoc. Amer. 44*, 15-18

132. Bamforth, C. (2008) Grape versus Grain. Cambridge University Press

133. Bamforth, C.W. (2009). Beer and the Quality of Life. Proc IBD Africa Convention (no page numbers).

134. Cluett, J., Heydenrych, M., Bamforth, C.W., Jackson, S., Lamaletie, B., Eloff, K. and Golob, H. (2009) Beer is food – a recipe to sustain the brewing industry in South Africa. *Chem. Tech*. August, 22-27

135. Bamforth, C.W. and Murphey, L.J. (2011) Beer and Health, in Beer Steward Handbook, Master Brewers Association of the Americas, pages 193-201

136. Bamforth, C. (2015) "Beer Is Good for You" as a Message in Academia, pp. 113-118 in "Ethanol and Education: Alcohol as a Theme for Teaching Chemistry", ed. R. Barth, American Chemical Society Symposium, http://pubs.acs.org/isbn/9780841230590

137. Bamforth, C. (2015) Respect for Beer. *Brew. Dist. Int. 11 (12*), 30-32

138. Wright, C.A., Bruhn, C.M., Heymann, H. and Bamforth, C.W. (2008) Beer and Wine Consumers' Perceptions of the Nutritional Value of Alcoholic and Non-alcoholic Beverages. *J. Food Sci. 73*, H8-H11. https://doi.org/10.1111/j.1750-3841.2007.00606.x

139. Wright, C.A., Bruhn, C.M., Heymann, H. and Bamforth, C.W. (2008) Beer Consumers' Perceptions of the Health Aspects of Alcoholic Beverages. *J. Food Sci. 73*, H12-H17. https://doi.org/10.1111/j.1750-3841.2007.00574.x

140. Smythe, J.E. and Bamforth, C.W. (2009) An evaluation of the public understanding of beer and brewing. *Tech. Quart. Mast. Brew. Assoc. Amer*. doi:10.1094/TQ-46-1-0316-01

141. DiPietro, M.B. and Bamforth, C.W. (2011) A comparison of the antioxidant potential of wine and beer. *J. Inst. Brew. 117*, 547-555. https://doi.org/10.1002/j.2050-0416.2011.tb00503.x

142. Owens, J.E., Clifford, A.J. and Bamforth, C.W. (2007) Folate in beer. *J. Inst. Brew. 113*, 243-248. https://doi.org/10.1002/j.2050-0416.2007.tb00283.x

143. Bamforth, C.W. (2012). Inorganic ions in beer – a survey. *Tech. Quart. Mast. Brew. Assoc. Amer*. *49*, 131-133.

144. Casey, T.R. and Bamforth, C.W. (2010) Silicon in Beer and Brewing. *J. Sci. Food Ag., 90*, 784–788. https://doi.org/10.1002/jsfa.3884

145. Guerdrum, L.J. and Bamforth, C.W. (2011). Levels of gliadin in commercial beers. *Food Chem. 129*, 1783-1784. ps://doi.org/10.1016/j.foodchem.2011.06.021

146. Guerdrum, L.J. and Bamforth, C.W. (2012). Prolamin levels through brewing and the impact of prolyl endopeptidase. *J. Am. Soc. Brew. Chem. 70*, 35-38. https://doi.org/10.1094/ASBCJ-2012-0130-01

147. Bamforth, C.W. (2005). Beer, carbohydrates and diet. *J. Inst. Brew. 111*, 259-264. https://doi.org/10.1002/j.2050-0416.2005.tb00681.x

148. Bamforth, C.W. (1999) Beer Haze. *J. Am. Soc. Brew. Chem. 57*, 81-90. https://doi.org/10.1094/ASBCJ-57-0081

149. Bamforth, C.W. (2011) 125th Anniversary Review: The non-biological instability of beer, *J. Inst. Brew. 117*, 488-497. https://doi.org/10.1002/j.2050-0416.2011.tb00496.x

150. Clark, D.T. and Bamforth, C.W. (2007) Realistic haze specifications for beer. *Tech. Quart. Mast. Brew. Assoc. Amer*. *44*, 160-163

151. Miedl, M. and Bamforth, C.W. (2004) The relative importance of temperature and time in the cold conditioning of beer. *J. Am. Soc. Brew. Chem. 62*, 75-78. https://doi.org/10.1094/ASBCJ-62-0075

152. Miedl, M., Garcia, M.A. and Bamforth, C.W. (2006). Haze formation in model beer systems. *J. Ag. Food Chem. 53*, 10161-10165. https://doi.org/10.1021/jf0506941

153. Mitchell, A.E., Hong, Y.-J., May, J.C., Wright, C.A. and Bamforth, C.W. (2005) A comparison of Polyvinylpolypyrrolidone (PVPP), Silica Xerogel and a Polyvinylpyrrolidone (PVP)-

866 silica co-product for their ability to remove polyphenols from beer. *J. Inst. Brew. 111*, 20-25.
867 https://doi.org/10.1002/j.2050-0416.2005.tb00644.x
868 154. Bamforth, C.W. and May, J.C. (2008) The recovery of polyphenols from PVPP and their
869 antioxidant capacity. *Tech. Quart. Mast. Brew. Assoc. Amer. 45*, 283–285
870 155. Bushnell, S.E., Guinard, J.-X. and Bamforth, C.W. (2003) Effects of sulfur dioxide and
871 polyvinylpolypyrrolidone (PVPP) on the flavor stability of beer as measured by sensory and
872 chemical analysis. *J. Am. Soc. Brew. Chem. 61*, 133-141. https://doi.org/10.1094/ASBCJ-61-0133
873 156. Bamforth, C.W. and Jackson, G. (1983) Anomalous haze readings due to β-glucans. *J.*
874 *Inst. Brew. 89*, 155-156. https://doi.org/10.1002/j.2050-0416.1983.tb04159.x
875 157. Bamforth, C.W., Butcher, K.N. and Cope, R. (1989) The interrelationships between
876 parameters of beer quality. *Ferment, 2*, 54-58
877 158. Smythe, J.E. and Bamforth, C.W. (2000) Shortcomings in Standard Instrumental Methods
878 for Assessing Beer Color. *J. Am. Soc. Brew. Chem*. *58*, 165-166. https://doi.org/10.1094/ASBCJ-
879 58-01650
880 159. Bamforth, C.W. and Simpson, W.J. (1995) Ionic equilibria in brewing*. Brew. Guard.*
881 *124(12),* 18-24
882 160. Bamforth, C.W. (2001) pH in brewing: an overview. *Tech. Quart. Mast. Brew. Assoc.*
883 *Amer. 38*, 1-9
884 161. Heisner, C.B. and Bamforth, C.W. (2008) Thioredoxin in barley: could it have a role in
885 releasing limit dextrinase in brewery mashes? J. Inst. Brew. 114, 122-126.
886 https://doi.org/10.1002/j.2050-0416.2008.tb00316.x
887 162. Bamforth, C.W. (2003) Barley and Malt Starch in Brewing: a general review. *Tech. Quart.*
888 *Mast. Brew. Assoc. Amer. 40*, 89-97

163. Hammond, J.R.M. and Bamforth, C.W. (1993) Progress in the development of new barley, hop and yeast variants for malting and brewing. *Biotech. Gen. Eng. Rev. 11*, 147-169. DOI: 10.1080/02648725.1993.10647900

164. Hammond, J.R.M. & Bamforth, C.W. (1994) Practical use of gene technology in food production. *Brewer*, 90, 65-69

165. Denby, C.M., Li, R.A., Vu, V.T., Costello, Z., Lin, W., Chan, L.J.D., Williams, J., Donaldson, B., Bamforth, C.W., Petzold, C.J., Scheller, H.V., Martin, H.G., and Keasling, J.D. (2018). Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. *Nature Comm. 9*, DOI: 10.1038/s41467-018-03293-x

166. Bokulich, N., Mills, D.A. and Bamforth, C.W. (2012). A review of molecular methods for microbial community profiling of beer and wine. *J. Am. Soc. Brew. Chem. 70*, 150-162. https://doi.org/10.1094/ASBCJ-2012-0709-01

167. Bokulich, N.A. and Bamforth, C.W. (2013). The Microbiology of Malting and Brewing. *Micro. Mol. Biol. Rev. 77*, 157-172. DOI: 10.1128/MMBR.00060-12

168. Bokulich, N.A. and Bamforth, C.W. (2017) eds. Brewing Microbiology: Current Research, Omics and Microbial Ecology. Caister Academic Press, Norfolk, UK

169. Bokulich, N., Bamforth, C.W. and Mills, D.A. (2012). Brewhouse resident microbiota are responsible for multi-stage fermentation of American Coolship Ale. *PLoS ONE 7(4):* e35507. doi:10.1371/journal.pone.0035507

170. Domizio, P., House, J.F., Joseph, C.M.L., Bisson, L.F. and Bamforth, C.W. (2016), Lachancea thermotolerans as an alternative yeast for the production of beer. *J. Inst. Brew. 122*, 599-604. https://doi.org/10.1002/jib.362

171. Bamforth, C.W. (1985) The use of enzymes in brewing. *Brew. Guard. 114(9*), 21-26

172. Bamforth, C.W. (1986) Enzymes from the grist. *Brewer, 72*, 427-434

173. Bamforth, C.W. (1987) Enzyme additions: requirements, optimization and potential problems. *Proc. Eur. Brew. Conv. Wort Symp., Maffliers*, 149-162

174. Hegarty, P.K. and Bamforth, C.W. (1989) The evaluation of commercial enzymes in brewing. *Ferment,* 2, 296-299

175. Bamforth, C.W. (2000) Endogenous and Exogenous enzymes in malting and brewing. *Proceedings of the 2nd European Symposium on enzymes in grain processing*, 149-155

176. Bamforth, C.W. (2009) Use of exogenous enzymes in the production of alcoholic beverages. The Encyclopedia of Biotechnology in Agriculture and Food, Taylor and Francis

177. Bamforth, C. (2008) The ultimate enzymology: making beer. *Food Sci. Tech. 22(4*), 12-14

178. Bamforth, C.W. (2009) Current perspectives on the role of enzymes in brewing. *J. Cer. Sci. 50*, 353-357. https://doi.org/10.1016/j.jcs.2009.03.001

179. Cabras, I. and Bamforth, C. (2015) From reviving tradition to fostering innovation and changing marketing: The evolution of micro-brewing in UK and US 1980-2012. *Bus. Hist. 58* https://doi.org/10.1080/00076791.2015.1027692

180. Cabras, I. and Bamforth, C. (2015). Interesting times: Curses and Changes in Brewing, in Beer, Brewing and Pubs: A Global Perspective, ed D. Higgins, D. Preece and I. Cabras, Palgrave MacMillan

181. Bamforth, C. W. (2006). Don't like it – but hard to deny it. *Brew. Guard. 135(1*), 20-22

182. Bamforth, CW. (2017) Progress in Brewing Science and Beer Production. *Ann. Rev. Chem. Biomol. Eng. 8*: 161-176. https://doi.org/10.1146/annurev-chembioeng-060816-101450

183. Bamforth, C. (1999) Whither beer? *Ferment, 12 (6*), 9-12

184. Bamforth, C. (2001) Beer: A proud past and a promising future. *Brew. Int. 1 (2*), 26-29

185. Bamforth, C.W. (2003) Opportunities for newer technologies in the oldest biotechnology, brewing. *App. Biotech. Food Sci. Pol. 1,* 213-222.

186. Heymann H, Goldberg, JR, Wallin CE and Bamforth CW (2010) A “beer” made from a bland alcohol base*. J. Am. Soc. Brew. Chem. 68*, 75-76. https://doi.org/10.1094/ASBCJ-2010-0409-01

187. Russell, S.T., Singh, R.P and Bamforth, C.W. (2008) Alternative Paradigms for the Production of Beer. *J. Inst. Brew. 114*, 349–356. https://doi.org/10.1002/j.2050-0416.2008.tb00779.x

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960 Legend to Figure

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962 Fig 1. Models for the cell wall of barley. (a) the model after Bamforth and Kanauchi (30); (b) a revised
963 model to account for the presence of soluble β-glucan on the surface of the walls. In either case it
964 should be realised that the various layers represent long-chain fibrous molecules that have a degree of
965 porosity and are not “solid walls”. It is likely that there is intermingling of the various polymers.

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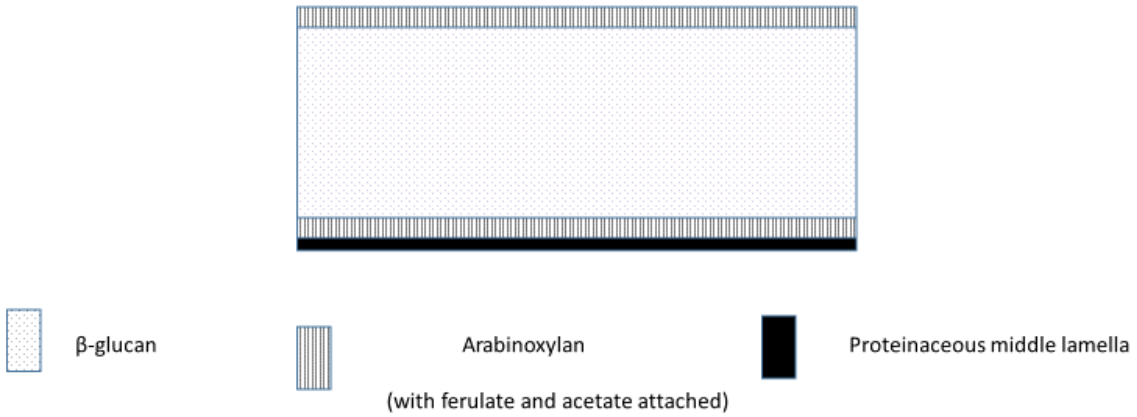
(a)



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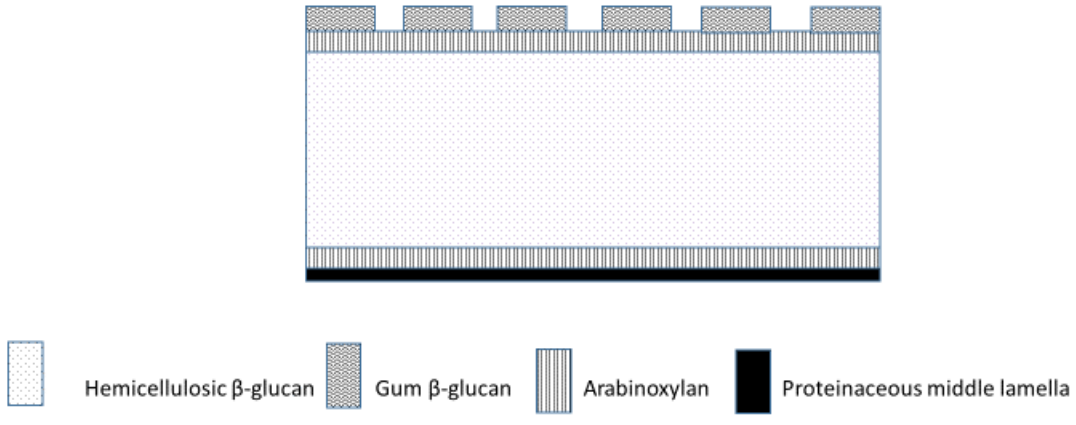
(b)



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